ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1987, p. 1194–1198
0066-4804/87/081194-05$02.00/0

Vol. 31, No. 8

Ranking of Prophylactic Efficacy of Poly(ICLC) against Rift Valley Fever Virus Infection in Mice by Incremental Relative Risk of Death

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Received 6 March 1987/Accepted 26 May 1987

The prophylactic efficacy of poly(ICLC) (stabilized, synthetic, double-stranded polyriboinosinic-polyribocytidylic acid) against Rift Valley fever virus infection in Swiss-Webster mice was dependent on the treatment schedule. The treatment schedule was optimized by ranking the results of various treatments by the Cox proportional-hazard model based on the incremental relative risk of death. With this ranking procedure, the schedule of choice was three doses of 20 μg each given 5 days apart. This regimen yielded a 90% survival rate. Additional parameters were determined, including the timing of the first and second drug dose, the temporal relationship of these treatments to the day of challenge, and the minimal effective dose (1 μg per mouse).

Poly(ICLC), the stabilized, synthetic, double-stranded polyriboinosinic-polyribocytidylic acid, is an effective in vivo immunomodulator and interferon inducer (2, 7, 9, 14). In primates and rodents, poly(ICLC) has proved useful in the prophylaxis of several viral infections (1, 3, 5, 8, 11, 12). Poly(ICLC) confers an antiviral state that persists for several days by stimulation of the immune response (2, 14) and induction of interferon production (7, 8). Interferon and the cellular immune response are both believed to play a role in altering the pathogenesis of viral infections.

The objectives of this study were to identify the optimal prophylactic treatment schedule of poly(ICLC) in Rift Valley fever virus (RVFV)-infected mice, delineate the optimal timing between the first and additional doses of drug, and determine the minimal effective dose as a guide for a possible human study. The efficacy of various poly(ICLC) treatment schedules was also ranked by a statistical model.

MATERIALS AND METHODS

Antiviral compounds. Poly(ICLC) was prepared by the Pharmaceutical Service, College of Pharmacy, University of Iowa, Iowa City. Each milliliter contained 2 mg of poly(ICLC), 1.5 mg of poly-L-lysine, and 5 mg of carboxymethylcellulose in a 0.9% sodium chloride solution. The pH was adjusted to 7.6 to 7.8 with sodium hydroxide. Treatment was by intraperitoneal (i.p.) injection.

Mice. Female Swiss-Webster mice, 8 to 10 weeks old, were purchased from Charles River Breeding Laboratories, Inc., Wilmington, Mass. The mice were observed for 3 weeks after viral challenge.

Virus. The Zagazig Hospital 501 strain of RVFV was isolated during the 1977 epidemic in Cairo, Egypt. The virus was grown on Vero cells and quantitated by a plaque assay. Briefly, virus was inoculated into 24-well culture plates containing 24-h, near-confluent Vero cells. To allow adsorption of the virus, cultures were incubated for 60 min at 37°C before addition of 0.5 ml of overlay medium (0.25% agarose in Eagle basal medium with Earle salt solution, supplemented with 16 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], 7.5% heat-inactivated fetal bovine serum, and 5 μg of gentamicin per ml). Cells were further incubated at 37°C for 96 h and stained with 0.1% crystal violet to make the plaques visible. In the in vivo efficacy studies, mice were injected subcutaneously in the left groin with 0.1 ml (250 PFU) of virus.

Statistical model. Determination of the optimal treatment schedule was done by ranking the treatment efficacy with the Cox proportional-hazard model, which defines efficacy in terms of the incremental relative risk of death compared with the relative risk of death of the standard day 0 treatment (4). The Cox model uses information from the entire survival curve to estimate the hazard of mortality as a function of the treatment schedule. The mathematical basis of the model is given by the equation $H(T,Z) = g(T)\exp(bZ)$, where T is time and Z is the indicator variable for schedule, $H(T,Z)$ is the hazard (instantaneous rate of death) at time T for an animal in schedule Z, $g(T)$ is the underlying hazard function for the standard schedule ($Z = 0$), and b is the regression coefficient for schedule Z.

The factor $\exp(bZ)$ is the incremental relative risk of death (relative to the standard, defined as $Z = 0$; in our case, treatment and challenge at day 0). A test of $b = 0$ determines whether the incremental relative risk of death is significantly different from that of day 0 standard treatment, either increasing the risk ($b > 0$) or decreasing the risk ($b < 0$) relative to the standard. Special statistical methods were used to fit the model by using software from the Biomedical Data Processing computer package (4).

RESULTS

Determination of optimal prophylactic treatment schedule. In the exploratory prophylactic study, eight or seven doses of 20 μg of poly(ICLC) per mouse were administered from day −4 until day +10 or from day −1 until day +10, respectively (Fig. 1). Although these multiple-dose treatment schedules yielded 100% and 90% long-term survivors, respectively, practical purposes dictated the determination of the least number of injections that yielded a satisfactory survival rate.

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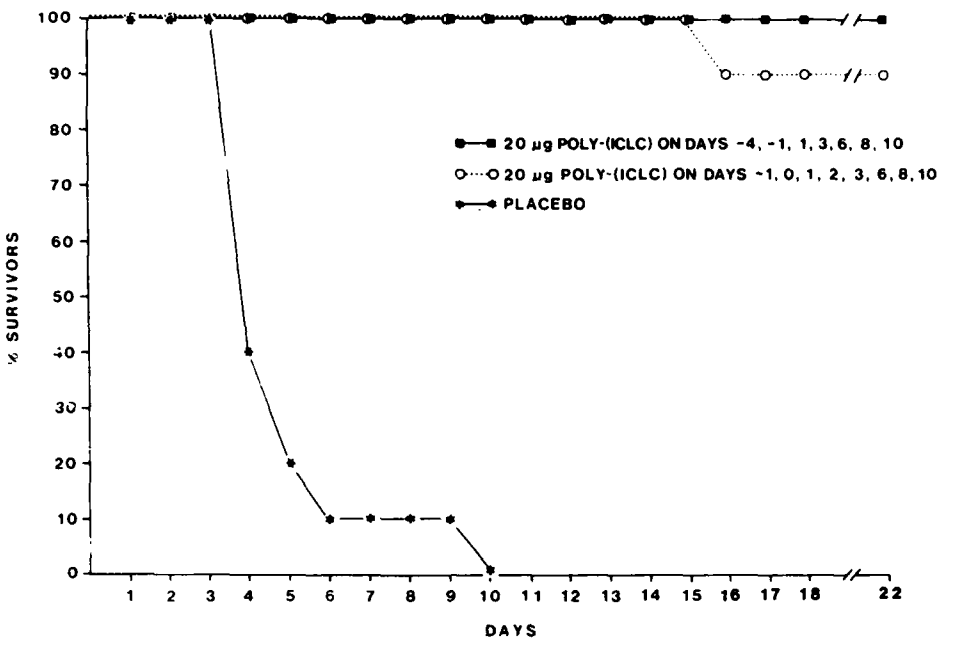

FIG. 1. Prophylactic efficacy of multiple doses of poly(ICLC). The mice (n = 10) were challenged subcutaneously on day 0 with 250 PFU of RVFV and injected i.p. with 20 μg of poly(ICLC) per mouse as indicated. Placebo was administered on days −4, −1, +1, +3, +6, and +8.

To determine the response to single inoculations at a fixed dose, groups of mice were treated once with 20 μg of poly(ICLC) 1, 2, 3, 4, or 5 days prior to challenge with 250 PFU of RVFV on day 0, simultaneously with the viral challenge, or 1, 2, or 3 days after challenge (Fig. 2). Treatment on day 0 gave the best overall efficacy, although treatment on day −1 was almost as effective. Treatment on earlier days yielded proportionally decreased efficacy. Even a single treatment 1 day postinfection considerably prolonged survival time compared with untreated control mice, which all died by day 4.

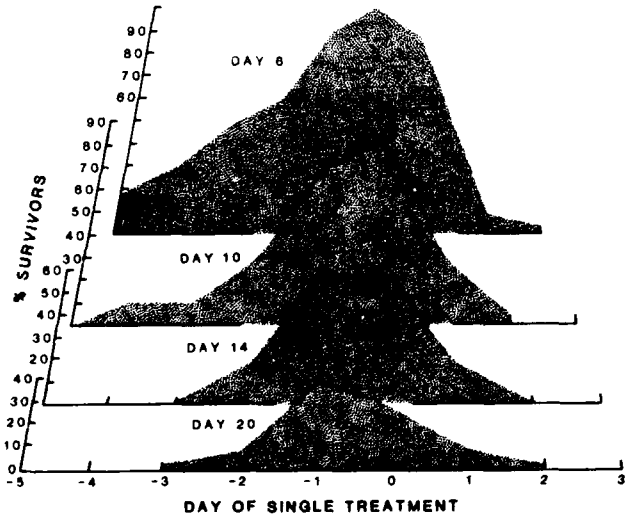

FIG. 2. Efficacy of a single dose of poly(ICLC). The mice (n = 10) were challenged subcutaneously on day 0 with 250 PFU of RVFV and injected once i.p. with 20 μg of poly(ICLC) 1, 2, 3, 4, or 5 days prior to challenge, on day 0, or 1, 2, or 3 days after challenge. The number of survivors in the treatment groups was scored on days 6, 10, 14, and 20.

The optimal treatment schedule was determined by ranking the treatment efficacy with the Cox proportional-hazard model (Table 1) (4). The table shows the schedules of single, double, and triple treatments, chosen to span a range of inoculation schedules between 5 days pre- and 3 days postchallenge. The number of survivors at day 20 is also shown. The relative risk of death associated with each treatment schedule is listed in increasing or decreasing order in reference to the relative risk of death of standard day 0 treatment, to which a value of 1.00 was assigned. The respective P values for the relative risk of death are also listed. The ranking created three categories: treatment schedules which were inferior to the standard (day 0) treatment because of their significantly higher ($P < 0.05$) relative risk of death (for example, when treatment was administered on day −5, the relative risk of death was 7.81 times higher than that for day 0 treatment); treatment schedules for which the relative risk of death was indistinguishable from that seen with day 0 treatment; and treatment schedules for which the relative risk of death was 5 to 10 times less than that seen with the day 0 treatment, indicating significantly ($P < 0.05$) greater efficacy. The two-dose treatment schedule (days −1 and +1) was undefined because of the proximity of the two doses, even though 100% of the mice on this schedule were long-term survivors. The ranking procedure indicated that the schedule of choice required treatment on days −4, +1, and +6. This treatment schedule represented the greatest interval between injections of poly(ICLC) that provided protection to 90% of treated mice. All of the schedules highlighted in the lower third of the table resulted in comparable treatment efficacies.

The survival data at day 10 (Fig. 2) indicated that a single treatment with 20 μg of poly(ICLC) on day 0 prolonged the life of all the infected mice by 6 days in comparison with untreated mice (data not shown), which delineates the opti-

TABLE 1. Efficacy ranking of various treatment regimens versus standard treatment in RVFV-infected mice

Treatment day(s)[a]	No. of survivors (n = 10)	Relative risk of death	Efficacy vs standard treatment
+3	0	38.07	Significantly lower ($P < 0.05$)
+2	0	21.19	
−5	0	7.81	
−4, +4	0	6.42	
−3	0	4.96	
−5, +5	1	4.47	
−4	1	4.33	
−3, +3	0	4.12	
−2	1	2.69	
+1	1	2.39	Indistinguishable ($P \geq 0.05$)
−2, +3	2	1.35	
−3, +1	2	1.14	
−2, +2	3	1.05	
0[b]	3	1.00	
−1	4	0.92	
−1, +6, +11	7	0.39	
−1, +4	8	0.36	
−2, +2, +4	8	0.22	
0, +4	8	0.21	Significantly higher ($P < 0.05$)
−3, +1, +5	8	0.21	
−1, +1, +3	9	0.11	
−4, +1, +6	9	0.11	
0, +4, +7	9	0.10	
−1, +1	10	Undefined	

[a] Treatments that put mice at significantly less risk are highlighted by boldfacing.
[b] Standard treatment.

mal spacing of two-dose or three-dose treatment schedules. The effect of a single treatment given on day 0 diminished by day 10 and thereafter (Fig. 2); therefore, to minimize the risk of death, the interval between treatments should not exceed 5 days. Furthermore, the timing of the first treatment relative to the viral challenge determined the required timing for administration of the second and third doses of poly(ICLC) (Fig. 3). If the first treatment was close to the viral challenge time, the second treatment could be delayed. The reverse was true only if a third dose was administered, because giving the second treatment close to viral challenge did not provide prolonged protection.

Determination of the minimal effective dose. To avoid possible toxic complications with a dose of 20 μg of poly(ICLC), the minimal effective dose was determined by administering poly(ICLC) on two schedules (Fig. 4). On these schedules, doses of 20, 10, 5, 1, and 0.5 μg of poly(ICLC) per mouse were administered i.p. against 250 PFU of RVFV. No significant differences were observed in the efficacy (ranging between 70 and 100%) obtained with 20-, 10-, 5-, and 1-μg doses on both schedules used. The dose of 0.5 μg of poly(ICLC) per mouse was marginally effective when given on days −4, +1, and +6 but was not effective when given on the days −1 and +1 schedule (40 and 20% survivors, respectively).

DISCUSSION

The prophylactic, antiviral efficacy of an immunodulator lasts longer than that of a conventional antiviral compound. The former induces interferon and a variety of cellular responses which are responsible for the antiviral state. However, after providing the primary stimulus, the inducer is not required as long as the induced antiviral state exists. A single administration of poly(ICLC) was sufficient to prolong the survival time of mice infected with RVFV for several days. In contrast, the activity of an antiviral compound usually does not last longer than several hours and is dependent on the presence of the drug in the serum and in certain organs.

Although a single dose of poly(ICLC) prolonged survival time, administration of multiple doses was required for complete protection. Empirical determination of an optimal,

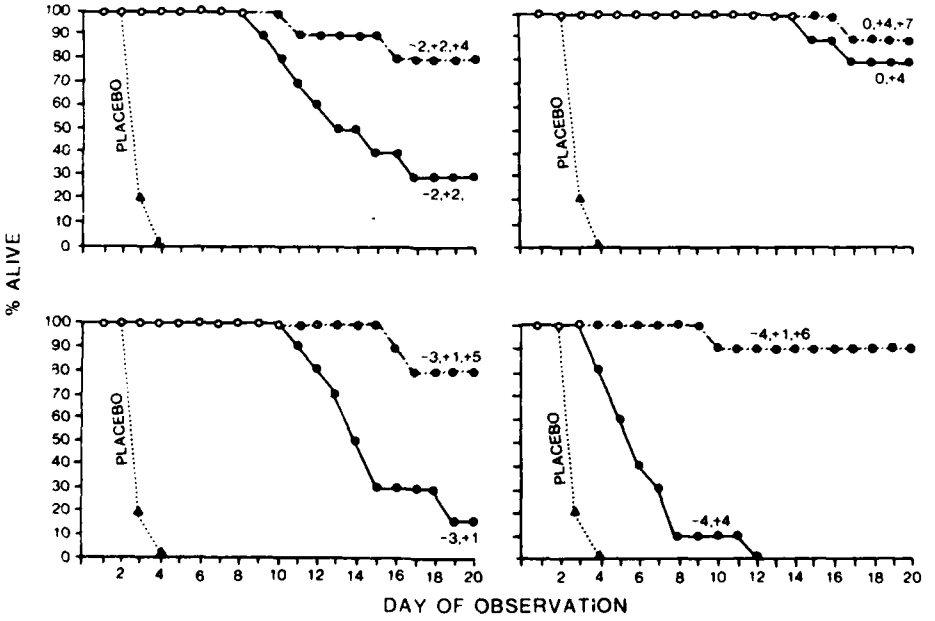

FIG. 3. Comparative therapeutic dose effects of poly(ICLC). The mice (n = 10) were challenged subcutaneously on day 0 with 250 PFU of RVFV and injected i.p. with 20 μg of poly(ICLC) per mouse on the days indicated on the curves.

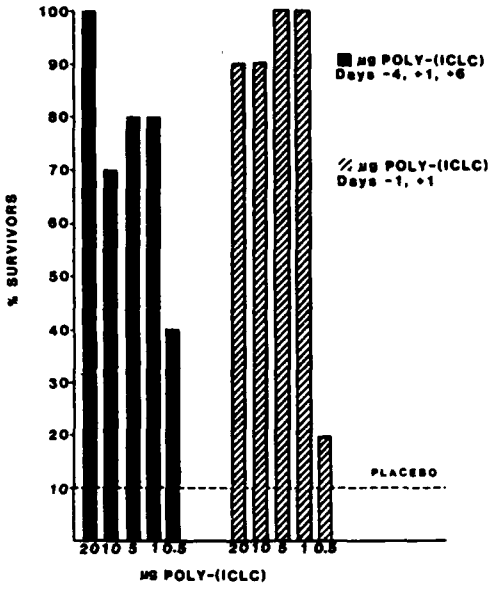

FIG. 4. Minimal effective dose of poly(ICLC). The mice (n = 10) were challenged subcutaneously on day 0 with 250 PFU of RVFV and injected i.p. with 20, 10, 5, 1, or 0.5 μg of poly(ICLC) per mouse on the indicated schedules.

multiple-dose drug treatment schedule is very tedious unless the optimal schedule can be estimated with the help of a statistical model. Even though a single dose of poly(ICLC) given prior to viral challenge was sufficient to protect 40% of the infected mice, this effect diminished when the time interval between treatment and challenge was increased. Nevertheless, increased survival time was observed even when a single dose of the compound was administered 5 days before RVFV challenge.

By using single treatments between days −5 and +3, we obtained a functional relationship between time of treatment and survival that was most apparent on days 6 and 10 (Fig. 2). The functional relationship between the time of the treatment and the survival pattern permitted the ranking of treatment efficacies according to the incremental relative risk of death. With the ranking procedure of Cox, the efficacy of a two-dose treatment schedule was estimated. Therefore, instead of 45 groups of mice, only 16 groups were required to test all possible one-dose and two-dose treatment schedules between day −5 and day +3. For example, any treatment schedule with doses spaced 6 or more days apart probably will not be effective unless the first treatment is administered 1 or 2 days prior to the challenge. Treatments spaced 5 and 4 days apart will yield efficacies equal to that of the standard treatment. Treatment schedules with doses spaced less than 4 days apart are expected to be in the effective treatment category. The borderlines between the categories were distinctive statistically; however, the values above or below the borderline did not clearly differ biologically and could have occurred in either one of the groups. Therefore, the values highlighted in Table 1 were selected as optimal treatment schedules because they were distinctly different from the borderline values.

This model permitted selection of the optimal treatment schedule. Effective treatment was obtained even when treatment was initiated 4 days prior to viral challenge and was followed with two doses given 5 days apart. The relatively long intervals between administration of the compound were consistent with the long-lasting immunomodulatory effect of poly(ICLC) (M. Kende et al., manuscript in preparation). A single administration of poly(ICLC) provided protection for several days, a second dose extended the protection, and a third dose prevented relapse.

Poly(ICLC) is well tolerated in mice. In 20-g male and female Swiss-Webster mice, the 50% lethal dose for a single dose was 20 and 18 mg/kg, respectively (H. Levy, unpublished data). In the present study, the highest dose tested was 0.8 mg/kg. Prophylactically, as little as 1 μg of poly(ICLC) per mouse, equivalent to 40 μg/kg, was effective against RVFV infection. A dose of 0.5 μg was less effective, although when it was administered three times a substantial number of the treated mice were protected from lethal RVFV infection (M. Kende, unpublished observation). Therefore, the minimal effective dose in this virus-host model is 1 μg per mouse. In comparison with 20 μg of poly(ICLC) per mouse, 1 μg induced 10 times less interferon (Kende et al., in preparation). In other studies, it was shown that 1 μg of poly(ICLC) was as effective as 20 μg in stimulating natural killer cells and macrophage cytotoxic activity (13). These cell-mediated activities have a potential role in inducing an antiviral state. The antiviral efficacy of a low dose of poly(ICLC) renders this compound a prime candidate for human use, particularly because of its broad spectrum of activity. The use of a broad-spectrum antiviral drug is advantageous when the identity of the virus is unknown or when the properties of the virus are altered and fast intervention is critical for survival, although under these circumstances, such use will be limited to unique military situations. It is conceivable that prophylactic antiviral immunity can be maintained with poly(ICLC) for a long period of time by administering the compound at 5- to 6-day intervals, thereby continuously protecting humans against a potential viral threat. While poly(ICLC) at higher doses can exert significant undesirable side effects in humans (6), doses analogous to 1 μg per mouse have been safely administered for the treatment of multiple sclerosis (10). Assessment of the minimal effective dose of poly(ICLC) in large groups of patients with multiple sclerosis is in progress (A. Salazar, personal communication).

ACKNOWLEDGMENTS

We appreciate the skillful typing of the manuscript by Marcia Baker and Molly Shepley-Stone and the editorial work by Katheryn Kenyon.

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